Title

Triglyceride-Rich Lipoprotein Cholesterol and Risk of Cardiovascular Events Among Patients Receiving Statin Therapy in the TNT Trial.

Journal

Circulation, 138(8)

ISSN

0009-7322

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Publication Date

2018-08-01

DOI

10.1161/circulationaha.117.032318

Peer reviewed

Circulation

ORIGINAL RESEARCH ARTICLE

Triglyceride-Rich Lipoprotein Cholesterol and Risk of Cardiovascular Events Among Patients Receiving Statin Therapy in the TNT Trial

BACKGROUND: Mendelian randomization data suggest that the genetic determinants of lifetime higher triglyceride-rich lipoprotein-cholesterol (TRL-C) are causally related to cardiovascular disease and therefore a potential therapeutic target. The relevance of TRL-C among patients receiving statins is unknown. We assessed the relationship between TRL-C and cardiovascular risk, and whether this risk was modifiable among patients receiving statins in the TNT trial (Treating to New Targets).

METHODS: Patients with coronary heart disease and low-density lipoprotein cholesterol (LDL-C) 130 to 250 mg/dL entered an 8-week run-in phase with atorvastatin 10 mg/d (ATV10). After this period, participants with LDL-C <130 mg/dL entered the randomized phase with ATV10 (n=5006) versus atorvastatin 80 mg/d (ATV80, n=4995). The primary end point was coronary heart disease death, nonfatal myocardial infarction, resuscitated cardiac arrest, or stroke (major adverse cardiovascular events [MACE]). TRL-C was calculated as total cholesterol minus high-density lipoprotein cholesterol minus LDL-C. The effect of atorvastatin on TRL-C was assessed during the run-in phase (ATV10) and randomized phase (ATV80 versus ATV10). The risk of MACE was assessed across quintiles (Q) of baseline TRL-C (and, for comparison, by baseline triglycerides and non–high-density lipoprotein cholesterol) during the randomized period. Last, the association between TRL-C changes with atorvastatin and cardiovascular risk was assessed by multivariate Cox regression.

RESULTS: ATV10 reduced TRL-C 10.7% from an initial TRL-C of 33.9±16.6 mg/dL. ATV80 led to an additional 15.4% reduction. Cardiovascular risk factors positively correlated with TRL-C. Among patients receiving ATV10, higher TRL-C was associated with higher 5-year MACE rates (Q1=9.7%, Q5=13.8%; hazard ratio Q5-versus-Q1, 1.48; 95% confidence interval, 1.15–1.92; *P*-trend<0.0001). ATV80 (versus ATV10) did not significantly alter the risk of MACE in Q1-Q2, but significantly reduced risk in Q3-Q5 (relative risk reduction, 29%–41%; all *P*<0.0250), with evidence of effect modification (*P*-homogeneity=0.0053); results were consistent for triglycerides (*P*-homogeneity=0.0101) and directionally similar for non–high-density lipoprotein cholesterol (*P*-homogeneity=0.1387). Last, in adjusted analyses, a 1 SD percentage reduction in TRL-C with atorvastatin resulted in a significant lower risk of MACE (hazard ratio, 0.93; 95% confidence interval, 0.86–1.00; *P*=0.0482) independent of the reduction in LDL-C and of similar magnitude to that per 1 SD lowering in LDL-C (hazard ratio, 0.89; 95% confidence interval, 0.83–0.95; *P*=0.0008).

CONCLUSIONS: The present post hoc analysis from TNT shows that increased TRL-C levels are associated with an increased cardiovascular risk and provides evidence for the cardiovascular benefit of lipid lowering with statins among patients who have coronary heart disease with high TRL-C.

CLINICAL TRIAL REGISTRATION: URL: https://www.clinicaltrials.gov. Unique identifier: NCT00327691.

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Key Words: atorvastatin calcium ■ cardiovascular diseases ■ cholesterol, LDL ■ lipids ■ lipoproteins ■ remnant-like particle cholesterol ■ triglycerides

Sources of Funding, see page 779

https://www.ahajournals.org/journal/circ

Clinical Perspective

What Is New?

- Higher levels of triglyceride-rich lipoprotein-cholesterol (TRL-C) are associated with a significantly higher rate of cardiovascular events among coronary patients treated with statins.
- Statin therapy reduces TRL-C levels to a higher extent among those treated with a higher statin dose.
- We provide evidence from a randomized trial (post hoc analysis) that more intensive statin therapy with atorvastatin 80 mg, in comparison with atorvastatin 10 mg, results in a significantly greater cardiovascular risk reduction among patients with higher TRL-C levels. Results were consistent for higher triglycerides and directionally concordant for non–high-density lipoprotein cholesterol.
- A higher percentage reduction in TRL-C levels was associated with lower cardiovascular risk independently of low-density lipoprotein cholesterol reduction.

What Are the Clinical Implications?

- Our data support those from observational and genetic studies suggesting that TRL-C is a risk factor for cardiovascular disease.
- In addition, the present analysis suggests a cardiovascular benefit of lipid-lowering medication among those with higher TRL-C levels.
- Taken together, the present results suggest that TRL-C levels are not only a cardiovascular risk marker, but also a potential target for therapeutic intervention.
- These data provide the rationale for the development of drugs aiming to reduce triglycerides and TRL-C; that may include novel agents under development targeting angiopoietin-like 3 or apolipoprotein C3.

The total cholesterol content in blood can be simply separated into the cholesterol carried by high-density lipoproteins (HDL-C), which principally contain apolipoprotein A-I, or the remainder, which is carried by a range of atherogenic lipoproteins including low-density lipoprotein (LDL-C) and triglyceride-rich lipoproteins (TRL-C; also referred to in the literature as remnant cholesterol) (such as very low-density lipoproteins, intermediate-density lipoproteins, chylomicrons, and their remnants), which principally all contain apolipoprotein B (apoB) (Figure 1). The total cholesterol content of these apoB-containing atherogenic particles can be simply calculated by subtracting HDL-C from total cholesterol (non-HDL-C); non-HDL-C mostly reflects the cholesterol content of LDL; however, the contribution of the cholesterol carried by TRL particles to non-HDL-C can become substantial when triglyceride levels are high.[1,2]

At a population level, the rising tide of obesity and diabetes mellitus and their known association with hypertriglyceridemia may mean that risk algorithms based on LDL-C levels only may no longer be sufficient to appropriately measure the atherogenic lipid risk. Indeed, there is considerable evidence suggesting that non-HDL-C is more strongly associated with cardiovascular disease (CVD) than LDL-C, both in statin-naive and in statin-treated patients.[3,4] Furthermore, Mendelian randomization data suggest that TRL-C is a causal factor for CVD.[5–7] Together with advances in novel therapies (such as antisense to apolipoprotein C3 [APOC3] drugs, which target TRL-C),[8] there is considerable interest in whether TRL-C is simply a marker of risk or whether it is a modifiable target for therapeutic intervention.

Statins increase the clearance of apoB-containing lipoproteins and therefore are expected to not only reduce LDL-C levels, but also TRL-C levels. Using data from the TNT trial (Treating to New Targets), we assessed the relationship between baseline and changes in TRL-C and subsequent cardiovascular events. The aims of the present analysis specifically assess: (1) whether TRL-C is a good marker of risk, (2) whether any excess risk related to TRL-C is modifiable by more intensive statin therapy, and (3) whether TRL-C is a modifiable target for future therapies.

METHODS

The TNT trial has been described in detail elsewhere.[9,10] In brief, TNT enrolled 35- to 75-year-old men and women with clinically evident coronary heart disease (CHD) (defined as previous myocardial infarction, previous or present angina with objective evidence of atherosclerotic CHD, or a history of coronary revascularization), and fasting untreated (wash-out period where needed) LDL-C 130 to 250 mg/dL (3.4–6.5 mmol/L) plus triglycerides ≤600 mg/dL (≤6.8 mmol/L). Of the 18 469 individuals screened, 15 464 met the eligibility criteria and entered an 8-week open-label run-in phase where all participants were given atorvastatin 10 mg once daily; after this period, 10 001 participants with LDL-C <130 mg/dL (3.4 mmol/L) (on atorvastatin 10 mg), absence of adverse events, and compliant with the medication were subsequently entered the randomized phase and were allocated (double-blind) and received atorvastatin 10 mg (n=5006) or atorvastatin 80 mg (n=4995) once daily (Figure I in the online-only Data Supplement).

The TNT primary end point was the time to occurrence of a major adverse cardiovascular event (MACE), defined as the composite of CHD death, nonfatal non–procedure-related myocardial infarction, resuscitated cardiac arrest, or fatal or nonfatal stroke. Median follow-up was 4.9 years.

The TNT study was approved by the local research ethics committees or institutional review board at each center and all patients gave written informed consent.[9,10] The TNT trial was sponsored by Pfizer. The sponsor did not provide financial support for the present analysis. Pfizer's policies on the provision of clinical trial data are set out on their website.[11] In addition to posting clinical trial results on the clinicaltrials.gov registry, Pfizer will provide access to anonymized patient-level data in response to scientifically valid research protocols.

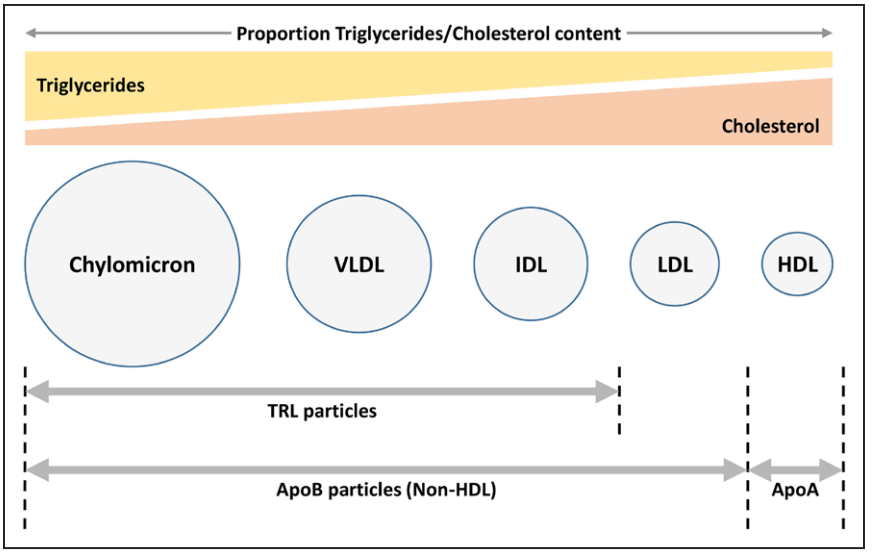


Figure 1. Lipoprotein particles.
ApoA indicates apolipoprotein A; ApoB, apolipoprotein B; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; TRL, triglyceride-rich lipoprotein; and VLDL, very low-density lipoprotein.

Present Analyses

Among TNT participants who entered the randomized trial (n=10 001), 9993 (99.9%) had data on TRL-C levels at baseline, ie, at the time of randomization (atorvastatin 10 mg, n=5003; atorvastatin 80 mg, n=4990) (Figure I in the online-only Data Supplement), and were included in the present analyses. TRL-C levels were calculated as the difference between fasting non-HDL-C and LDL-C. Non-HDL-C was defined as total cholesterol minus HDL-C. LDL-C was calculated using the Friedewald formula[12] (unless triglyceride levels were ≥400 mg/dL [≥4.5 mmol/L], where LDL-C was directly measured by ultracentrifugation). These calculated TRL-C levels have been reported to closely approximate the levels of triglycerides.[13]

In the present analyses, the effect of atorvastatin 10 mg on TRL-C levels was assessed during the open-label run-in phase, and that of atorvastatin 80 mg versus 10 mg over 5 years was assessed during the randomized phase, to determine if a dose-dependent effect on TRL-C levels exists. The risk of MACE was assessed across quintiles of baseline TRL-C levels during the randomized period. Last, observational analyses were performed to elucidate the extent to which the reductions achieved with atorvastatin in TRL-C levels influence outcomes.

Further details on methods are shown in Methods in the online-only Data Supplement and Figure I in the online-only Data Supplement.

Statistical Analysis

Mean TRL-C levels during the trial are shown as mean±SD and median, interquartile range. Patients were grouped by quintiles of TRL-C at baseline (ie, at the time of randomization). The characteristics of participants were compared across quintiles as follows: the Cochran-Mantel-Haenszel statistics (Cochran-Armitage Trend Test) were used for categorical variables, and regression analysis was conducted for continuous variables with quintile as a continuous variable in the model.

Effect of Statin Therapy on Outcomes

The randomized effect of atorvastatin 80 mg in comparison with atorvastatin 10 mg on the risk of MACE was assessed for each quintile of TRL-C at baseline. Absolute risk reductions and numbers needed-to-treat were calculated based on the event rates in both arms where appropriate. Estimates of hazard ratios (HRs) and 95% confidence intervals (95% CIs) with corresponding *P* values for each quintile were obtained by Cox-regression analysis, with randomized therapy as the only covariate. To assess whether the effect of atorvastatin 80 mg versus 10 mg was consistent across TRL-C quintiles, a test for interaction was performed (test for homogeneity). By way of comparison, the same estimates were obtained for quintiles of triglycerides and quintiles of non-HDL-C at baseline.

Changes in TRL-C Levels and On-Treatment TRL-C and Outcomes

The independent association between the absolute and relative change in TRL-C levels from baseline to 3 months and the on-treatment TRL-C levels with the subsequent cardiovascular risk were assessed by means of multivariable Cox-regression models. On-treatment TRL-C levels were defined as levels at month 3 after randomization. Participants with events before 3 months were excluded for these analyses. TRL-C levels were entered as log-transformed values (to normalize the distribution of the variable to include it in the model). Adjusted HR (95% CI) and corresponding *P* values were calculated per 1 SD change/on-treatment TRL-C. Multivariable models accounted for the following covariates: age, sex, smoking status, hypertension, diabetes mellitus, prior myocardial infarction, baseline log(TRL-C), LDL-C and HDL-C, and absolute change or percentage change or on-treatment (as appropriate) LDL-C and HDL-C levels. By way of comparison, the same estimates were obtained for LDL-C levels, and similar models were built for triglycerides (log-transformed) and for non-HDL-C levels (replacing TRL-C by the corresponding lipid fraction; in the case of non-HDL-C, LDL-C levels were not included in the models to avoid colinearity because LDL-C is contained in non-HDL-C).

The statistical analyses were performed using SAS software, versions 9.2/9.3 (SAS Institute Inc). Tests were 2-sided. Statistical significance was defined as $P<0.05$. Trial registration: ClinicalTrials.gov, NCT00327691.

RESULTS

Impact of Statins on TRL-C

Mean (±SD) TRL-C level at the beginning of the run-in phase was 33.9±16.6 mg/dL (0.9±0.4 mmol/L). Atorvastatin 10 mg reduced TRL-C levels during the run-in phase by a median of 10.7% (median absolute change, –3.0 mg/dL [–0.1 mmol/L]; interquartile range, –10.0 to 3.0 mg/dL [–0.3 to 0.1 mmol/L]; *P*<0.0001). This reduction remained similar throughout the study in those allocated to atorvastatin 10 mg during the randomized period (Figure 2). In those allocated to atorvastatin 80 mg after the initial run-in phase with 10 mg, TRL-C was reduced by a further 15.4% (median absolute change, –4.0 mg/dL [–0.1 mmol/L]; interquartile range, –9.0 to 1.0 mg/dL [–0.2 to 0.0, mmol/L]; *P*<0.0001). The time-course levels of TRL-C during the randomized phase are shown in Figure 2. Median levels of TRL-C were lower in the group of patients allocated to atorvastatin 80 mg throughout the randomized trial (Figure 2 and Figure II in the online-only Data Supplement). The time-course levels of LDL-C by treatment arm are shown in Figure III in the online-only Data Supplement.

Demographics Across TRL-C Quintiles

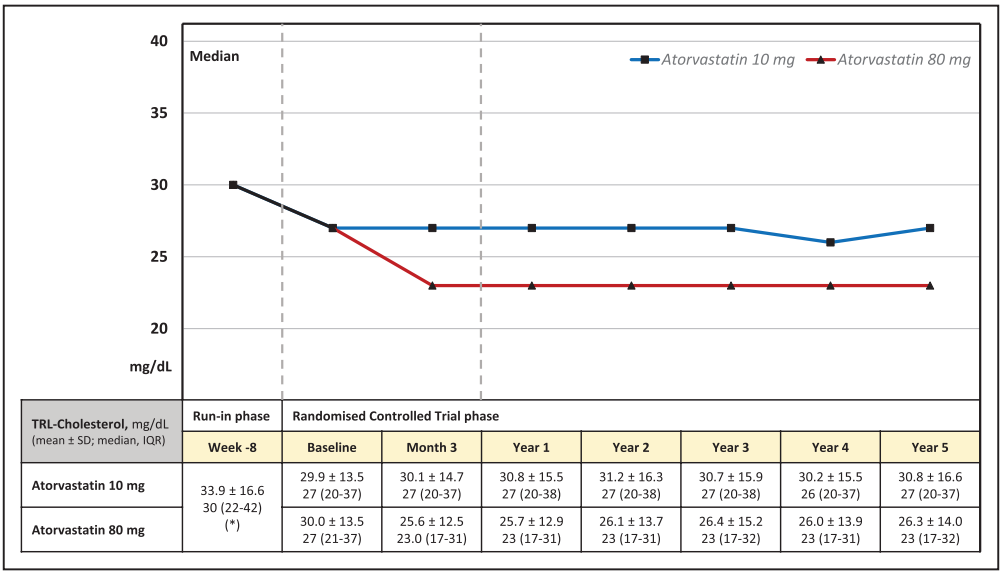
The characteristics of participants by TRL-C quintiles at baseline are shown in the Table (the quantiles corresponded to TRL-C levels of 19.0, 24.0, 30.0, and 39.5 mg/dL [0.5, 0.6, 0.8, and 1.0 mmol/L]). Patients in lower quintiles were slightly older and more frequently men than those in higher quintiles (62.0±8.6 years and 86.6% men in quintile 1 versus 59.5±8.9 years and 76.6% men in quintile 5; *P*<0.0001 for trend). It is notable that the presence of cardiovascular risk factors such as hypertension, diabetes mellitus and levels of fasting glucose, smoking, body mass index, and an unfavorable lipid profile (higher total cholesterol, LDL-C, non-HDL-C and triglycerides, and lower HDL-C) positively correlated with levels of TRL-C (all *P*<0.0001 for trend across quintiles). Although LDL-C levels were significantly higher for quintile 5 than for quintile 1 (mean 99.1±18 versus 94.2±17 mg/dL [2.6±0.5 versus 2.4±0.4 mmol/L]), this difference was substantially less marked than that observed for non-HDL-C (150.8±21 versus 109.9±17 mg/dL [3.9±0.5 versus 2.8±0.4 mmol/L]) or for TRL-C (51.7±10.8 versus 15.7±2.5 mg/dL [1.3±0.3 versus 0.4±0.1 mmol/L]).

TRL-C Levels and Outcomes

Among patients receiving atorvastatin 10 mg, higher TRL-C levels were associated with higher rates of MACE at 5 years, ranging from 9.7% in quintile 1 to 13.8% in quintile 5; HR for quintile 5 versus quintile 1, 1.48 (95% CI, 1.15–1.92); *P* for trend quintile 1 to quintile 5 <0.0001 (Figure 3). Among patients receiving atorvastatin 80 mg, baseline TRL-C levels were not associated with outcomes (*P* for trend quintile 1 to quintile 5, *P*=0.7806; Table I in the online-only Data Supplement).

Effect of Statin Therapy on Outcomes

Figure 4A depicts the effect of atorvastatin 80 mg versus 10 mg on the risk of MACE by quintiles of TRL-C at baseline. In the lowest 2 quintiles there was no significant benefit from atorvastatin 80 mg on cardiovascular outcomes in comparison with atorvastatin 10 mg. However, with increasing levels of TRL-C there was evidence of effect modification (*P*-homogeneity 0.0053)



TRL-Cholesterol, mg/dL (mean ± SD; median, IQR)	Run-in phase	Randomised Controlled Trial phase						
	Week -8	Baseline	Month 3	Year 1	Year 2	Year 3	Year 4	Year 5
Atorvastatin 10 mg	33.9 ± 16.6 30 (22-42) (*)	29.9 ± 13.5 27 (20-37)	30.1 ± 14.7 27 (20-37)	30.8 ± 15.5 27 (20-38)	31.2 ± 16.3 27 (20-38)	30.7 ± 15.9 27 (20-38)	30.2 ± 15.5 26 (20-37)	30.8 ± 16.6 27 (20-37)
Atorvastatin 80 mg		30.0 ± 13.5 27 (21-37)	25.6 ± 12.5 23.0 (17-31)	25.7 ± 12.9 23 (17-31)	26.1 ± 13.7 23 (17-31)	26.4 ± 15.2 23 (17-32)	26.0 ± 13.9 23 (17-31)	26.3 ± 14.0 23 (17-32)

Figure 2. Time course levels of triglyceride-rich lipoprotein (TRL) cholesterol grouped by treatment arm.
Data shown as mean± SD and median (interquartile range [IQR]). *All patients received atorvastatin 10 mg/d during the 8-week run-in phase. To convert cholesterol values from mg/dL to mmol/L, multiply by 0.02586. TRL indicates triglyceride-rich lipoprotein.

Table. Baseline Characteristics of Participants, by Quintiles of Triglyceride-Rich Lipoprotein Cholesterol at Baseline

	Triglyceride-Rich Lipoprotein Cholesterol, by Baseline Quintiles					
	≤19.0 mg/dL	>19.0–24.0 mg/dL	>24.0–30.0 mg/dL	>30.0–39.5 mg/dL	>39.5 mg/dL	
	n=2125	n=1951	n=1991	n=1966	n=1960	*P* Value
Age, y	62.0±8.6	61.6±8.8	61.6±8.7	60.3±8.9	59.5±8.9	<0.0001
Sex: male	1820 (85.6)	1619 (83.0)	1608 (80.8)	1544 (78.5)	1501 (76.6)	<0.0001
Hypertension	1017 (47.9)	974 (49.9)	1126 (56.5)	1093 (55.6)	1200 (61.2)	<0.0001
Diabetes mellitus	228 (10.7)	225 (11.5)	280 (14.1)	347 (17.6)	420 (21.4)	<0.0001
Current smokers	260 (12.2)	251 (12.9)	225 (11.3)	287 (14.6)	316 (16.1)	<0.0001
Prior myocardial infarction	1253 (59.0)	1141 (58.5)	1145 (57.5)	1150 (58.5)	1138 (58.1)	0.9081
Body mass index, kg/m^2	27.0±3.9	27.8±4.2	28.6±4.8	29.3±4.6	30.0±4.7	<0.0001
Glucose, mg/dL	102.8±25.6	103.7±24.9	106.4±27.6	110.6±33.3	115.5±38.0	<0.0001
Total cholesterol, mg/dL	163.0±21.6	168.7±21.2	171.7±20.9	178.5±20.8	192.9±23.1	<0.0001
HDL cholesterol, mg/dL	53.0±12.0	49.4±10.9	46.9±9.9	44.7±9.4	42.0±8.7	<0.0001
LDL cholesterol, mg/dL	94.2±16.7	97.5±17.6	97.7±17.1	99.2±17.7	99.1±18.5	<0.0001
Non–HDL cholesterol, mg/dL	109.9±17.1	119.2±17.6	124.8±17.3	133.8±17.9	150.8±21.5	<0.0001
Triglycerides, mg/dL	80.5 (70.0–89.0)	108.5 (102.5–115.0)	135.5 (128.0–143.0)	172.0 (160.5–184.5)	244.5 (217.5–287.8)	<0.0001
TRL cholesterol, mg/dL	15.7±2.5	21.7±1.4	27.1±1.7	34.6±2.7	51.7±10.8	<0.0001

Data shown as absolute and relative (%) numbers for qualitative variables; quantitative variables are shown as mean±SD, or as median and interquartile range, as appropriate. To convert cholesterol and triglycerides values from mg/dL to mmol/L, multiply by 0.02586 and 0.01129, respectively. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and TRL, triglyceride-rich lipoprotein.

with significant risk reductions observed in quintiles 3 to 5. Absolute risk reductions of 2.4%, 4.7%, and 3.9% were observed in quintiles 3, 4, and 5, respectively, corresponding to 5-year numbers needed-to-treat of 42, 21, and 26, respectively.

Changes in TRL-C Levels and Outcomes

In fully adjusted analyses, the percentage reduction in TRL-C from baseline to 3 months was associated with the risk of MACE independent of the reduction in LDL-C (Figure 5). The benefit observed with a 1 SD percentage lowering of log(TRL-C) (HR, 0.929; *P*=0.0482; 1 SD=9.0 [raw value=30.2]) was independent of LDL-C and was of similar magnitude to that associated with a 1 SD percentage lowering of LDL-C (HR, 0.890; *P*=0.0008; 1 SD=22.1) in models that contained both TRL-C and LDL-C. For comparison, the analysis of absolute change in levels of log(TRL-C) and on-treatment log(TRL-C) levels and risk of outcomes provided qualitatively similar findings, although these were not statistically significant (HR, 0.934, *P*=0.0650, and HR 0.900, *P*=0.0650, respectively) (Figure 5).

Comparative Analyses With Triglycerides and Non-HDL-C Replacing TRL-C

Baseline TRL-C levels correlated well with triglycerides and non-HDL-C levels (Pearson correlation coefficients: log[TRL-C] and log[triglycerides]: *r*=0.99, *P*<0.001; log[TRL-C] and non-HDL-C: *r*=0.64, *P*<0.001).

In analyses of risk of MACE with more versus less intensive statin therapy by baseline quintiles of triglycerides and treatment interactions, we observed a similar relationship as with TRL-C, with a significant *P* value for interaction of *P*=0.0101 (Figure 4B). Analyses of risk by baseline quintiles of non-HDL-C showed results directionally similar to those from TRL-C and triglycerides, although the magnitude of effect was somewhat attenuated and there was no interaction by treatment (*P*=0.1387) (Figure 4C).

In analysis assessing reductions in triglycerides, we observed qualitatively similar percentage risk reductions as with TRL-C (although in the case of triglycerides, these risk reductions did not reach the nominal level of statistical significance when LDL-C was included in the models; percentage change, absolute change, or on-treatment triglycerides levels, all per 1 SD lower in log[triglycerides]: HR 0.93 [*P*=0.0562], HR 0.94 [*P*=0.0784], and HR 0.91 [*P*=0.0784], respectively; Figure IV in the online-only Data Supplement). By way of comparison, analyses based on reductions in non-HDL-C levels led to stronger and significant risk reductions (HRs ranging from 0.84 to 0.87 per 1 SD lower of non-HDL-C, all *P*<0.0001; Figure V in the online-only Data Supplement).

DISCUSSION

The present analysis from the TNT trial in ≈10 000 patients with CHD receiving atorvastatin suggests a greater benefit of lipid-lowering therapy on cardiovascular

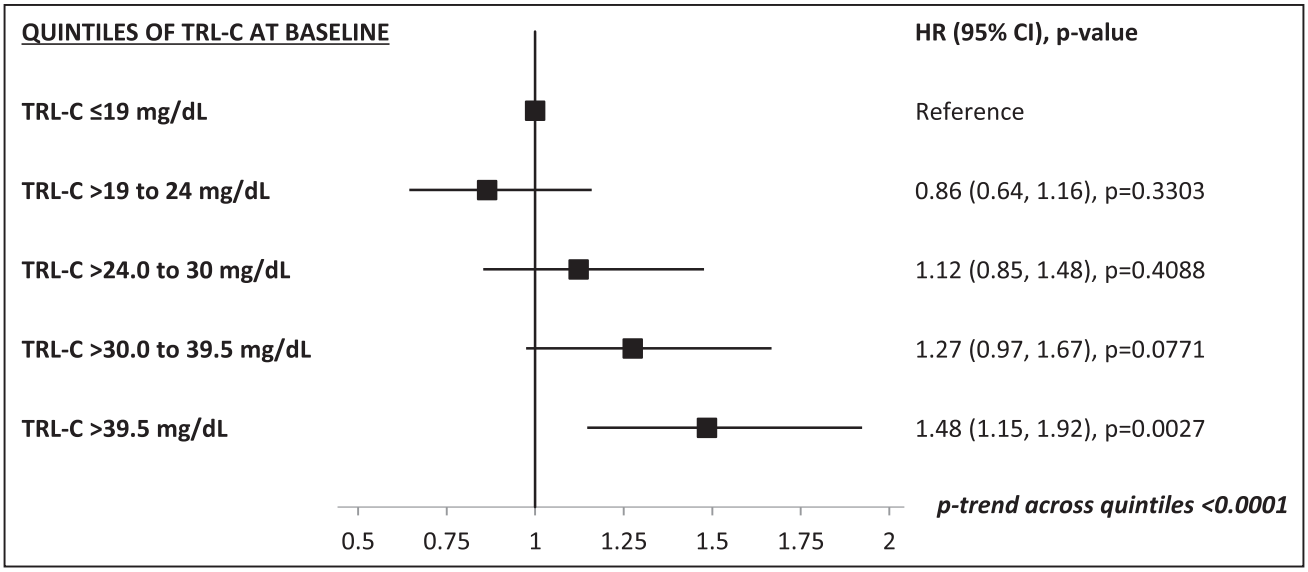


Figure 3. Risk of major cardiovascular events among patients receiving atorvastatin 10 mg based on quintiles of triglyceride-rich lipoprotein cholesterol at baseline.

To convert cholesterol values from mg/dL to mmol/L, multiply by 0.02586. CI indicates confidence interval; HR, hazard ratio; and TRL-C, triglyceride-rich lipoprotein cholesterol.

outcomes among those with higher TRL-C levels. The present analyses revealed that (1) higher TRL-C levels were associated with a higher prevalence of cardiovascular and metabolic risk factors; (2) treatment with atorvastatin significantly reduced TRL-C levels, to a greater extent in those treated with the highest statin dose; (3) higher TRL-C levels were associated with a higher rate of cardiovascular events at 5 years; (4) evidence from a randomized trial that more intensive statin therapy with atorvastatin 80 mg, in comparison with atorvastatin 10 mg, resulted in a significantly greater cardiovascular risk reduction among those patients with higher TRL-C levels (those in TRL-C quintiles 3–5 versus 1–2; significant interaction of statin intensity by TRL-C quintiles); the results for this analysis were consistent for TRL-C and triglyceride levels, and directionally similar for non-HDL-C; (5) observational evidence that a 1 SD percentage reduction in TRL-C levels with atorvastatin was associated with a lower risk of MACE independent of and of similar magnitude to a 1 SD reduction in LDL-C levels.

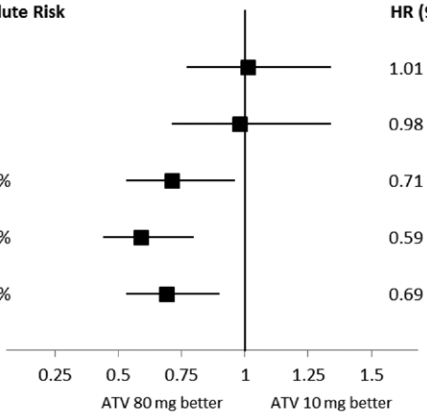
The present results fit well with previous reports from the TNT trial. For instance, patients with metabolic syndrome in the TNT trial have been observed to obtain more benefit from high-dose atorvastatin than did patients without metabolic syndrome,[14] which likely track with higher levels of TRL-C (and non-HDL-C and triglycerides) among those subjects with metabolic syndrome. These results are also concordant with the increased risk associated with higher triglyceride levels in the combined cohorts from the TNT trial and the IDEAL trial (Incremental Decrease in End Points through Aggressive Lipid Lowering).[15] Of interest, on the other hand, the present analysis revealed differences between treatment groups (statistically significant treatment-by-quintile interaction) when the data were stratified by TRL-C or triglyceride quintiles. This is unlike previous analysis from the TNT trial, which did not find differences between groups (no interaction) based on LDL-C levels.[16] In the same direction, in the Cholesterol Treatment Trialists meta-analysis, the relative risk reduction of major vascular events with statins per 39 mg/dL increase in LDL-C remained constant among different strata based on triglycerides levels.[17] Thus, our results support the notion that intensive lipid-lowering medication among those with higher TRL-C is of benefit for CV risk reduction; whether further intensive lipid lowering, eg, with PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors, leads to further benefit based on baseline TRL-C levels requires further research.

Despite optimal therapy with high-intensity statin regimes to lower LDL-C,[10,18] and, more recently, with the combination therapy of statins with ezetimibe[19] or PCSK9 inhibitors[20,21] to further lower LDL-C levels, a significant residual risk of CVD still persists. TRL-C may account, at least in part, for this residual risk. In fact, in our study, among coronary patients with an initial LDL-C <130 mg/dL (3.4 mmol/L) who were subsequently treated with atorvastatin 10 mg, we observed a significant higher risk of cardiovascular events with higher TRL-C levels (HR for quintile 5 versus quintile 1, 1.48; 95% CI, 1.15–1.92). Among participants in the high-intensity statin group (atorvastatin 80 mg), where a similar relationship was not observed among quintiles of TRL-C, TRL-C levels might be unlikely to predict future risk, because at least part of the excess risk (residual risk) is attenuated by receiving high-intensity therapy. In

A – TRL-C

QUINTILES OF TRL-C AT BASELINE	5-year Event Rate (%) ATV 80mg	ATV 10mg	Absolute Risk	HR (95% CI), p-value
TRL-C ≤19 mg/dL	9.76%	9.72%		1.01 (0.77, 1.34), p=0.9360
TRL-C >19 to 24 mg/dL	8.20%	8.62%		0.98 (0.71, 1.34), p=0.8809
TRL-C >24.0 to 30 mg/dL	8.21%	10.58%	-2.38%	0.71 (0.53, 0.96), p=0.0243
TRL-C >30.0 to 39.5 mg/dL	7.36%	12.07%	-4.71%	0.59 (0.44, 0.80), p=0.0006
TRL-C >39.5 mg/dL	9.84%	13.77%	-3.92%	0.69 (0.53, 0.90), p=0.0059

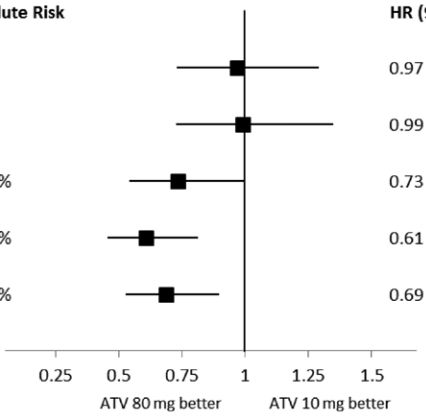
p-homogeneity = 0.0053



B - TRIGLYCERIDES

QUINTILES OF TG AT BASELINE	5-year Event Rate (%) ATV 80mg	ATV 10mg	Absolute Risk	HR (95% CI), p-value
TG ≤94.5 mg/dL	9.61%	9.92%		0.97 (0.73, 1.29), p=0.8385
TG >94.5 to 120 mg/dL	8.31%	8.61%		0.99 (0.73, 1.35), p=0.9476
TG >120 to 150 mg/dL	7.94%	10.12%	-2.18%	0.73 (0.54, 0.99), p=0.0449
TG >150 to 198 mg/dL	7.81%	12.35%	-4.53%	0.61 (0.46, 0.81), p=0.0008
TG >198 mg/dL	9.77%	13.68%	-3.91%	0.69 (0.53, 0.90), p=0.0055

p-homogeneity = 0.0101



C – NON-HDL-C

QUINTILES NON-HDL-C AT BASELINE	5-year Event Rate (%) ATV 80mg	ATV 10mg	Absolute Risk	HR (95% CI), p-value
Non-HDL-C ≤108 mg/dL	7.53%	8.70%		0.89 (0.65, 1.21), p=0.4465
Non-HDL-C >108 to 120 mg/dL	8.48%	9.18%		0.88 (0.65, 1.20), p=0.4239
Non-HDL-C >120 to 132 mg/dL	8.48%	10.67%		0.78 (0.59, 1.05), p=0.1006
Non-HDL-C >132 to 146 mg/dL	8.98%	12.86%	-3.88%	0.68 (0.51, 0.89), p=0.0061
Non-HDL-C >146 mg/dL	9.99%	13.35%	-3.36%	0.71 (0.54, 0.92), p=0.0112

p-homogeneity = 0.1387

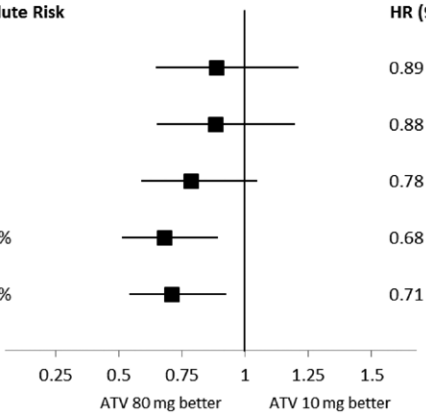


Figure 4. Effect of atorvastatin 80 mg versus atorvastatin 10 mg on the risk of major cardiovascular events by quintiles of triglyceride-rich lipoprotein cholesterol (A), triglycerides (B), and non-HDL-C (C) at baseline.

To convert cholesterol values from mg/dL to mmol/L, multiply by 0.02586. To convert triglyceride values from mg/dL to mmol/L, multiply by 0.01129. ATV indicates atorvastatin; CI, confidence interval; HR, hazard ratio; non-HDL-C, non–high-density lipoprotein cholesterol; TG, triglycerides; and TRL-C, triglyceride-rich lipoprotein cholesterol.

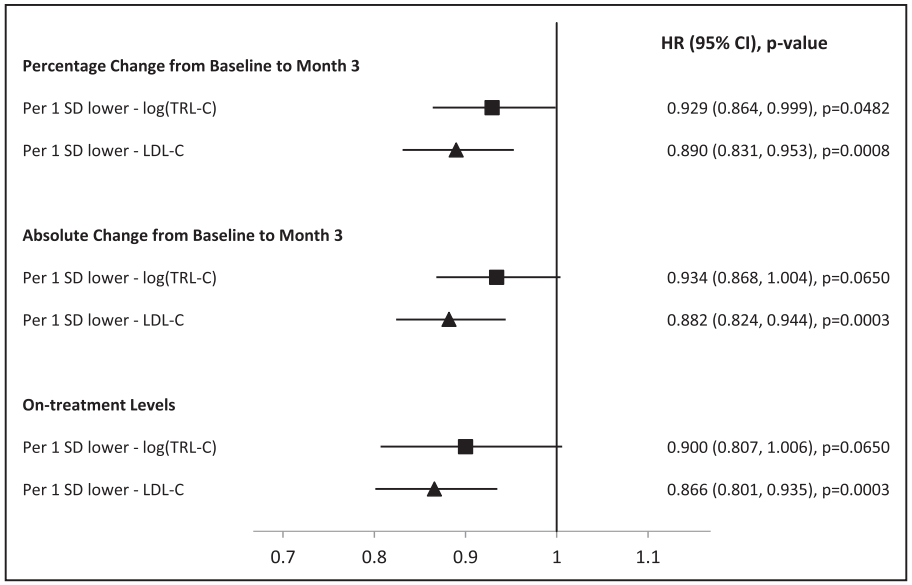


Figure 5. Association between TRL-C and LDL-C level reduction or on-treatment TRL-C and LDL-C levels and risk of major cardiovascular events. On-treatment levels are defined as levels at 3 months of therapy (randomized trial). The analysis excludes patients with events before 3 months. A 1 SD of percentage change in log(TRL-C) and of absolute change in log(TRL-C) from baseline to month 3 corresponds to 9.0 and 0.3, respectively (raw [log back] values: 30.2 and 9.6, respectively). A 1 SD of on-treatment log(TRL-C) levels corresponds to 0.4 (raw [log back] value, 13.8). The corresponding values for a 1 SD percentage change and absolute change in LDL-C levels and on-treatment LDL-C levels are 22.1, 21.4, and 24.5, respectively. Multivariate analysis adjusted for age, sex, smoking, hypertension, diabetes mellitus, myocardial infarction, baseline log(TRL-C), baseline LDL-C, baseline high-density lipoprotein cholesterol, and changes or on-treatment lipid levels as appropriate. Number of patients with on-treatment TRL-C data: atorvastatin 10 mg, n=4895; atorvastatin 80 mg, n=4874. To convert cholesterol and triglyceride values from mg/dL to mmol/L, multiply by 0.02586 and 0.01129, respectively. LDL-C indicates low-density lipoprotein cholesterol; and TRL-C, triglyceride-rich lipoprotein cholesterol.

contrast, among patients on atorvastatin 10 mg, higher TRL-C levels may represent a clinical group with higher residual risk in need of further lipid-lowering therapy. These results are in agreement with those reported by different observational studies, where elevated TRL-C levels have been associated with increased cardiovascular risk, mainly with CHD.[2,22] For instance, fasting TRL-C has recently been associated with the presence and severity of coronary artery calcium score independently of classical risk factors, including HDL-C and LDL-C, in a cohort of >3800 asymptomatic subjects without history of CVD from the ELSA-Brasil Study (Brazilian Longitudinal Study of Adult Health).[22] In addition, observational data from a large cohort of >73 500 subjects from the general population in Denmark reported an increased risk for CHD with increasing levels of TRL-C (P<0.001 for trend from quintile 1 to quintile 5)[2]; it is interesting to note that HRs were significant only among those within higher levels of TRL-C in comparison with those in the lower stratum (quintiles 4 and 5 [TRL-C >27 mg/dL, >0.7 mmol/L] versus quintile 1 [<15 mg/dL, <0.4 mmol/L]); HRs 2.0 [95% CI, 1.5–2.6] and 2.3 [95% CI, 1.7–3.1], respectively), but not among those in quintiles 2 and 3 versus quintile 1. These data are in the same direction as the results we have found in our study, where the cardiovascular benefit of more intensive statin therapy reached significance among those within quintiles 3 to 5 only (TRL-C >24.0 mg/dL [>0.6 mmol/L], thus with a threshold similar to that in the Danish study). More recently, an association of increased remnant cholesterol levels with all-cause mortality in patients with CHD has also been reported from the Copenhagen Ischemic Heart Disease Study (unlike increased measured LDL-C levels).[23]

In addition to observational studies suggesting a positive association between TRL-C and cardiovascular risk, a number of genetic studies has strongly supported higher TRL-C or triglyceride levels to be a causal risk factor for CVD.[24] For instance, Mendelian randomization analysis from the aforementioned Danish cohort found a significant 2.8-fold causal risk for CHD per 39 mg/dL (1.0 mmol/L) increase in nonfasting TRL-C, independent of LDL-C or HDL-C, and higher than that observed per similar increase in LDL-C.[2] Different genetic variants have further supported these observations. This is the case for the loss-of-function mutations in genes encoding ANGPTL3 (angiopoietin-like 3) and ANGPTL4 (angiopoietin-like 4) proteins[25,26] or loss-of-function or missense mutations in the APOC3 gene[6]; these genetic variants have been associated with substantially decreased levels of triglycerides, and carriers of these mutations have an associated lower rate of ischemic coronary or vascular disease.[5,6,25,26] Thus, these genetic variants related to lower levels of triglycerides seem to confer reduced genetic risk for CVD, a likely causal relationship.

The present analysis from the TNT trial complements and adds to the current available information from ob-

servational and genetic studies supporting the role of TRL-C on cardiovascular risk, by providing novel evidence from a randomized trial, not available so far, on the benefit of lipid-lowering medication among those with higher TRL-C. As such, therapy with atorvastatin 80 mg, in comparison with atorvastatin 10 mg, led to a significant 5-year relative risk reduction of MACE among those with TRL-C >24.0 mg/dL (0.6 mmol/L) ranging from 29% to 41%, which corresponded to absolute risk reductions of 2.4% to 4.7%. These results were consistent with those observed using triglyceride levels and directionally similar for non-HDL-C levels (see discussion below). Among the overall cohort of patients, the risk reduction associated with a 1 SD percentage reduction of TRL-C levels from baseline corresponded to a HR of 0.929 (P=0.0482), which was independent of LDL-C levels and comparable to that risk reduction achieved with a 1 SD percentage reduction in LDL-C (HR 0.890, P=0.0008). In similar analysis, reductions in non-HDL-C levels led to slightly stronger risk reductions than TRL-C and comparable to LDL-C (HR 0.866 per 1 SD percentage reduction in non-HDL-C, P<0.0001), in line with previous reports where non-HDL-C has been strongly associated with cardiovascular events (even stronger than LDL-C levels) among patients treated with statins.[4]

The mechanism by which elevated TRL-C leads to more MACE and why reducing it is associated with lower rates of MACE merit consideration. Figure 6 shows causal pathways by which LDL particles (cholesterol content of LDL) and triglyceride-related pathways (whether through triglycerides or TRL-C) increase cardiovascular risk. Our data cannot separate whether triglycerides or TRL-C is the causal factor of this triglyceride-related pathway (because TRL-C, as calculated from other lipid fractions, closely approximates to triglycerides divided by 5); however, biologically, triglycerides have not been directly implicated in the process of atherosclerosis, whereas cholesterol is clearly causal.[27] It has been suggested that, when plasma TRL-C is elevated, more TRLs enter from plasma into the arterial intima, which in turn lead to uptake and degradation of TRLs by monocyte-macrophages and consequently increase the cholesterol deposits in the atherosclerotic plaques; such inflamed plaques are more likely to rupture and cause MACE.[24] The role of TRL-C as an important factor in atherosclerotic CVD is also supported by the stronger risk reductions for non-HDL-C that contains both LDL-C and TRL-C in comparison with LDL-C. This is consistent with other analyses from statin trials.[4]

The relationship of TRL-C levels with atherosclerotic CVD risk is of particular importance considering the rise in prevalence of cardiometabolic risk conditions such as diabetes mellitus, obesity, or metabolic syndrome, which are frequently associated with a characteristic dyslipidemia that includes increased TRL-C levels along with high triglycerides and non-HDL-C and low HDL-C.[28] This has led to a renewed interest in medication that may effectively reduce triglycerides and TRL-C levels, and the present results from the TNT trial provide a rationale for this type of intervention. That includes novel agents under development targeting ANGPTL3 (human monoclonal antibody against Angptl3, evinacumab, or antisense oligonucleotides against Angptl3 messenger-RNA)[25,29] or APOC3 (antisense inhibitor of APOC3 synthesis).[8] The first observations in humans have demonstrated that these agents substantially reduce the levels of triglycerides in comparison with placebo.[8,25,29] Whether this effect will translate into the reduction of cardiovascular risk has not been addressed by the current available studies.

To date, there are no large randomized controlled trial data that have convincingly showed that lowering triglycerides reduces cardiovascular events, apart from a

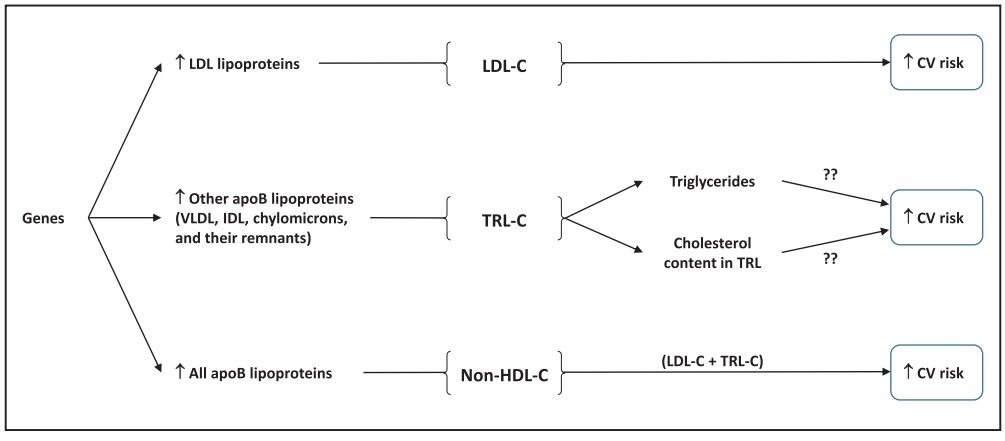


Figure 6. Causal pathways by which LDL particles (cholesterol content of LDL) and triglyceride-related pathways (whether through triglycerides or TRL-C) increase cardiovascular risk.

ApoB indicates apolipoprotein B; CV, cardiovascular; IDL, intermediate-density lipoprotein; non-HDL-C, non–high-density lipoprotein cholesterol; LDL(-C), low-density lipoprotein (cholesterol); TRL(-C), triglyceride-rich lipoprotein (cholesterol); and VLDL, very low-density lipoprotein.[4,24,27]

meta-analysis of randomized trials of fibrates versus placebo that reported a reduction of CHD events by 35% with fibrates among patients with high triglycerides and low HDL-C.[30] Several randomized clinical trials aiming specifically at patients with hypertriglyceridemia are currently ongoing, using omega-3 therapies (REDUCE-IT [Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial],[31] STRENGTH [Outcomes Study to Assess Statin Residual Risk Reduction With EpaNova in High CV Risk Patients With Hypertriglyceridemia][32]) or the novel selective peroxisome proliferator-activated receptor alpha modulator pemafibrate (PROMINENT [Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides in Patients With Diabetes][33]). These trials may provide further insights regarding the cardiovascular benefit of triglycerides and TRL-C lowering (independently of LDL-C) or non-HDL-C lowering on cardiovascular outcomes. With respect to PCSK9 inhibitors, they have shown in randomized trials of patients with high/not-on-target LDL-C, usually on top of statins, to substantially reduce apoB levels by ≈50% in comparison with controls, but their effect on triglyceride levels is far more limited[34]; to what extent this translates into TRL-C reduction (the cholesterol carried by apoB-related particles, and so by TRL-C, could be reduced by increasing the clearance of these particles),[35] and the latter into cardiovascular risk reduction, is currently under investigation.

Some limitations of the present analysis require consideration. For instance, although these analyses are from a randomized trial, they are post hoc analyses. The analyses based on changes in TRL-C levels and on-treatment TRL-C are observational in nature. Where applicable, the analyses have been adjusted for different baseline characteristics, although we cannot fully rule out the potential for confounding factors affecting the results. TRL-C levels were estimated from the levels of other lipid fractions as total cholesterol minus HDL-C minus LDL-C, where LDL-C was mostly estimated by the Friedewald formula, with no direct measurement of TRL-C; these calculated TRL-C levels have been reported to closely approximate the total content of triglycerides in blood and suggested that, in practice, the associations found might also be primarily with triglycerides[13]; this aligns with the strong correlation observed between calculated TRL-C and triglyceride levels in our study (r=0.99, P<0.001) and the results reported in a previous study by Varbo et al[2] (R^2 0.96, P<0.001); nevertheless, this methodology has been previously reported in prior studies and, more important, this calculated TRL-C has been related to CVD in different studies as previously discussed.[2,22,23] In the future it will be possible to measure TRL-C directly to further validate these findings.[36] Last, our analyses are based on fasting TRL-C levels; thus, although we do not expect our results to materially change and be similarly applicable to TRL-C levels measured during the nonfasting state (because they are in the same direction as those studies using nonfasting TRL-C), we cannot confirm they can be fully extrapolated to nonfasting TRL-C.

In summary, the present analysis from the randomized TNT trial shows that TRL-C levels are reduced by atorvastatin therapy in a dose-dependent fashion. Although higher TRL-C levels are associated with increased CVD risk, our results provide evidence for this cardiovascular risk to be significantly attenuated by intensive atorvastatin therapy in those with higher TRL-C levels (with consistent results for higher triglyceride levels and directionally concordant relationship for higher non-HDL-C levels). Independent of the reduction in LDL-C, the percentage reduction in TRL-C was associated with cardiovascular events. Taken together, these data suggest that TRL-C levels are both a risk marker and a potential target for therapeutic intervention.

ARTICLE INFORMATION

Received October 19, 2017; accepted March 21, 2018.

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The online-only Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/CIRCULATIONAHA.117.032318.

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Sources of Funding

The TNT trial (Treating to New Targets) was sponsored by Pfizer. The sponsor did not provide financial support for the present analysis.

Disclosures

Dr Vallejo-Vaz reports nonfinancial support from Regeneron and honoraria for lecture from Amgen, outside the submitted work. Dr Fayyad is a Pfizer employee. Dr Boekholdt has served on advisory boards for Pfizer and Sanofi-Aventis. Dr Hovingh is a recipient of a Vidi grant (project number 016.156.445) from the Netherlands Organisation for Scientific Research (NWO) and the European Union (Transcard FP7-603091-2); Dr Hovingh has served as a consultant and/or speaker for Pfizer, AMGEN, Regeneron, Sanofi, and Aegerion; fees for these services are invoiced by his institute and financial resources are exploited for research purposes. Dr Kastelein reports consultancy from Pfizer, Amgen, Sanofi-Regeneron, Astra-Zeneca, Esperion, Gemphire, and Madrigal. Dr Melamed is a Pfizer employee. Dr Barter reports grants and/or personal fees from Pfizer, Merck, Sanofi, and Amgen, outside the submitted work. Dr Waters reports remuneration for participating in clinical trial committees: CSL Ltd, the Medicines Company, Pfizer, Regeneron, Resverlogix, Sanofi; honoraria for lectures: Pfizer; consulting fees: Pfizer. Dr Ray reports grants and/or personal fees from Pfizer, MSD, Astra Zeneca, Sanofi, Aegerion, Regeneron, Abbvie, Kowa, Cerenis, Medicines Company, Lilly, Esperion, Amgen, Cipla, and Algorithm, outside the submitted work.

REFERENCES

1. Mark L, Vallejo-Vaz AJ, Reiber I, Paragh G, KondapallySeshasai SR, Ray KK. Non-HDL cholesterol goal attainment and its relationship with triglyceride concentrations among diabetic subjects with cardiovascular disease: a nationwide survey of 2674 individuals in Hungary. *Atherosclerosis*. 2015;241:62–68. doi: 10.1016/j.atherosclerosis.2015.04.810
2. Varbo A, Benn M, Tybjærg-Hansen A, Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. *J Am Coll Cardiol*. 2013;61:427–436. doi: 10.1016/j.jacc.2012.08.1026
3. Emerging Risk Factors Collaboration, Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, Collins R, Thompson SG, Danesh J. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA*. 2009;302:1993–2000. doi: 10.1001/jama.2009.1619
4. Boekholdt SM, Arsenault BJ, Mora S, Pedersen TR, LaRosa JC, Nestel PJ, Simes RJ, Durrington P, Hitman GA, Welch KM, DeMicco DA, Zwinderman AH, Clearfield MB, Downs JR, Tonkin AM, Colhoun HM, Gotto AM Jr, Ridker PM, Kastelein JJ. Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. *JAMA*. 2012;307:1302–1309. doi: 10.1001/jama.2012.366
5. TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute, Crosby J, Peloso GM, Auer PL, Crosslin DR, Stitziel NO, Lange LA, Lu Y, Tang ZZ, Zhang H, Hindy G, Masca N, Stirrups K, Kanoni S, Do R, Jun G, Hu Y, Kang HM, Xue C, Goel A, Farrall M, Duga S, Merlini PA, Asselta R, Girelli D, Olivieri O, Martinelli N, Yin W, Reilly D, Speliotes E, Fox CS, Hveem K, Holmen OL, Nikpay M, Farlow DN, Assimes TL, Franceschini N, Robinson J, North KE, Martin LW, DePristo M, Gupta N, Escher SA, Jansson JH, Van Zuydam N, Palmer CN, Wareham N, Koch W, Meitinger T, Peters A, Lieb W, Erbel R, Konig IR, Kruppa J, Degenhardt F, Gottesman O, Bottinger EP, O'Donnell CJ, Psaty BM, Ballantyne CM, Abecasis G, Ordovas JM, Melander O, Watkins H, Orho-Melander M, Ardissino D, Loos RJ, McPherson R, Willer CJ, Erdmann J, Hall AS, Samani NJ, Deloukas P, Schunkert H, Wilson JG, Kooperberg C, Rich SS, Tracy RP, Lin DY, Altshuler D, Gabriel S, Nickerson DA, Jarvik GP, Cupples LA, Reiner AP, Boerwinkle E, Kathiresan S. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med*. 2014;371:22–31. doi: 10.1056/NEJMoa1307095
6. Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjærg-Hansen A. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. *N Engl J Med*. 2014;371:32–41. doi: 10.1056/NEJMoa1308027
7. Jørgensen AB, Frikke-Schmidt R, West AS, Grande P, Nordestgaard BG, Tybjærg-Hansen A. Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. *Eur Heart J*. 2013;34:1826–1833. doi: 10.1093/eurheartj/ehs431
8. Gaudet D, Alexander VJ, Baker BF, Brisson D, Tremblay K, Singleton W, Geary RS, Hughes SG, Viney NJ, Graham MJ, Crooke RM, Witztum JL, Brunzell JD, Kastelein JJ. Antisense inhibition of apolipoprotein C-III in patients with hypertriglyceridemia. *N Engl J Med*. 2015;373:438–447. doi: 10.1056/NEJMoa1400283
9. Waters DD, Guyton JR, Herrington DM, McGowan MP, Wenger NK, Shear C; TNT Steering Committee Members and Investigators. Treating to New Targets (TNT) Study: does lowering low-density lipoprotein cholesterol levels below currently recommended guidelines yield incremental clinical benefit? *Am J Cardiol*. 2004;93:154–158.
10. LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, Gotto AM, Greten H, Kastelein JJ, Shepherd J, Wenger NK; Treating to New Targets (TNT) Investigators. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N Engl J Med*. 2005;352:1425–1435. doi: 10.1056/NEJMoa050461
11. Pfizer Trial Data & Results. http://www.pfizer.com/research/clinical_trials/trial_data_and_results. Accessed January 17, 2018.
12. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499–502.
13. Jones SR, Martin SS, Brinton EA. Letter by Jones et al regarding article, "Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation." *Circulation*. 2014;129:e655. doi: 10.1161/CIRCULATIONAHA.113.005954
14. Deedwania P, Barter P, Carmena R, Fruchart JC, Grundy SM, Haffner S, Kastelein JJ, LaRosa JC, Schachner H, Shepherd J, Waters DD; Treating to New Targets Investigators. Reduction of low-density lipoprotein cholesterol in patients with coronary heart disease and metabolic syndrome: analysis of the Treating to New Targets study. *Lancet*. 2006;368:919–928. doi: 10.1016/S0140-6736(06)69292-1
15. Faergeman O, Holme I, Fayyad R, Bhatia S, Grundy SM, Kastelein JJ, LaRosa JC, Larsen ML, Lindahl C, Olsson AG, Tikkanen MJ, Waters DD, Pedersen TR; Steering Committees of IDEAL and TNT Trials. Plasma triglycerides and cardiovascular events in the Treating to New Targets and Incremental Decrease in End-Points through Aggressive Lipid Lowering trials of statins in patients with coronary artery disease. *Am J Cardiol*. 2009;104:459–463. doi: 10.1016/j.amjcard.2009.04.008
16. Shepherd J, Barter P, Carmena R, Deedwania P, Fruchart JC, Haffner S, Hsia J, Breazna A, LaRosa J, Grundy S, Waters D. Effect of lowering LDL cholesterol substantially below currently recommended levels in patients with coronary heart disease and diabetes: the Treating to New Targets (TNT) study. *Diabetes Care*. 2006;29:1220–1226. doi: 10.2337/dc05-2465
17. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R, Simes R; Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*. 2005;366:1267–1278. doi: 10.1016/S0140-6736(05)67394-1
18. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ; JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359:2195–2207. doi: 10.1056/NEJMoa0807646
19. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, Darius H, Lewis BS, Ophuis TO, Jukema JW, De Ferrari GM, Ruzyllo W, De Lucca P, Im K, Bohula EA, Reist C, Wiviott SD, Tershakovec AM, Musliner TA, Braunwald E, Califf RM; IMPROVE-IT Investigators. Ezetimibe added to statin therapy after acute coronary syndromes. *N Engl J Med*. 2015;372:2387–2397. doi: 10.1056/NEJMoa1410489
20. Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, Stroes ES, Langslet G, Raal FJ, El Shahawy M, Koren MJ, Lepor NE, Lorenzato C, Pordy R, Chaudhari U, Kastelein JJ; ODYSSEY LONG TERM Investigators. Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372:1489–1499. doi: 10.1056/NEJMoa1501031
21. Sabatine MS, Giugliano RP, Wiviott SD, Raal FJ, Blom DJ, Robinson J, Ballantyne CM, Somaratne R, Legg J, Wasserman SM, Scott R, Koren MJ, Stein EA; Open-Label Study of Long-Term Evaluation against LDL Cholesterol (OSLER) Investigators. Efficacy and safety of evolocumab in reducing lipids and cardiovascular events. *N Engl J Med*. 2015;372:1500–1509. doi: 10.1056/NEJMoa1500858
22. Bittencourt MS, Santos RD, Staniak H, Sharovsky R, Kondapally R, Vallejo-Vaz AJ, Ray KK, Bensenor I, Lotufo P. Relation of fasting triglyceride-rich lipoprotein cholesterol to coronary artery calcium score (from the ELSA-Brasil Study). *Am J Cardiol*. 2017;119:1352–1358. doi: 10.1016/j.amjcard.2017.01.033
23. Jepsen AM, Langsted A, Varbo A, Bang LE, Kamstrup PR, Nordestgaard BG. Increased remnant cholesterol explains part of residual risk of all-cause mortality in 5414 patients with ischemic heart disease. *Clin Chem*. 2016;62:593–604. doi: 10.1373/clinchem.2015.253757
24. Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. *Lancet*. 2014;384:626–635. doi: 10.1016/S0140-6736(14)61177-6
25. Dewey FE, Gusarova V, Dunbar RL, O'Dushlaine C, Schurmann C, Gottesman O, McCarthy S, Van Hout CV, Bruse S, Dansky HM, Leader JB, Murray MF, Ritchie MD, Kirchner HL, Habegger L, Lopez A1, Penn J, Zhao A, Shao W, Stahl N, Murphy AJ, Hamon S, Bouzelmat A, Zhang R, Shumel B, Pordy R, Gipe D, Herman GA, Sheu WHH, Lee IT, Liang KW, Guo X, Rotter JI, Chen YI, Kraus WE, Shah SH, Damrauer S, Small A, Rader DJ, Wulff AB, Nordestgaard BG, Tybjærg-Hansen A, van den Hoek AM, Princen HMG, Ledbetter DH, Carey DJ, Overton JD, Reid JG, Sasiela WJ, Banerjee P, Shuldiner AR, Borecki IB, Teslovich TM, Yancopoulos GD, Mellis SJ, Gromada J, Baras A. Genetic and pharmacologic inactivation of ANGPTL3 and cardiovascular disease. *N Engl J Med*. 2017;377:211–221. doi: 10.1056/NEJMoa1612790
26. Folsom AR, Peacock JM, Demerath E, Boerwinkle E. Variation in ANGPTL4 and risk of coronary heart disease: the Atherosclerosis Risk in Communities Study. *Metabolism*. 2008;57:1591–1596. doi: 10.1016/j.metabol.2008.06.016

27. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, Hegele RA, Krauss RM, Raal FJ, Schunkert H, Watts GF, Borén J, Fazio S, Horton JD, Masana L, Nicholls SJ, Nordestgaard BG, van de Sluis B, Taskinen MR, Tokgözoglu L, Landmesser U, Laufs U, Wiklund O, Stock JK, Chapman MJ, Catapano AL. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur Heart J*. 2017;38:2459–2472. doi: 10.1093/eurheartj/ehx144
28. Borén J, Williams KJ. The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. *Curr Opin Lipidol*. 2016;27:473–483. doi: 10.1097/MOL.0000000000000330
29. Graham MJ, Lee RG, Brandt TA, Tai LJ, Fu W, Peralta R, Yu R, Hurh E, Paz E, McEvoy BW, Baker BF, Pham NC, Digenio A, Hughes SG, Geary RS, Witztum JL, Crooke RM, Tsimikas S. Cardiovascular and metabolic effects of ANGPTL3 antisense oligonucleotides. *N Engl J Med*. 2017;377:222–232. doi: 10.1056/NEJMoa1701329
30. Sacks FM, Carey VJ, Fruchart JC. Combination lipid therapy in type 2 diabetes. *N Engl J Med*. 2010;363:692–694; author reply 694.
31. Bhatt DL, Steg PG, Brinton EA, Jacobson TA, Miller M, Tardif JC, Ketchum SB, Doyle RT Jr, Murphy SA, Soni PN, Braeckman RA, Juliano RA, Ballantyne CM; REDUCE-IT Investigators. Rationale and design of REDUCE-IT: Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial. *Clin Cardiol*. 2017;40:138–148. doi: 10.1002/clc.22692
32. US National Institutes of Health. US National Library of Medicine. ClinicalTrials.gov. NCT02104817: Outcomes Study to Assess STatin Residual Risk Reduction WithEpaNova in HiGh CV Risk PatienTs With Hypertriglyceridemia (STRENGTH). https://clinicaltrials.gov/ct2/show/NCT02104817. Accessed July 31, 2017.
33. US National Institutes of Health. US National Library of Medicine. ClinicalTrials.gov. NCT03071692: Pemafibrate to Reduce Cardiovascular OutcoMes by Reducing Triglycerides IN patiENts With diabeTes (PROMINENT). https://clinicaltrials.gov/ct2/show/NCT03071692. Accessed 31 July, 2017.
34. Ray KK, Vallejo-Vaz AJ. The evolving role of CETP inhibition: beyond HDL cholesterol. *Lancet*. 2015;386:412–414. doi: 10.1016/S0140-6736(15)60608-0
35. Packard CJ. Unpacking and understanding the impact of proprotein convertase subtilisin/kexin type 9 inhibitors on apolipoprotein B metabolism. *Circulation*. 2017;135:363–365. doi: 10.1161/CIRCULATIONAHA.116.025897
36. Varbo A, Freiberg JJ, Nordestgaard BG. Remnant cholesterol and myocardial infarction in normal weight, overweight, and obese individuals from the copenhagen general population study. *Clin Chem*. 2018;64:219–230. doi: 10.1373/clinchem.2017.279463